



UNIVERSITI PUTRA MALAYSIA

**NUTRIENT COMPOSITION, ANTIOXIDANT AND ANTIPROLIFERATIVE
PROPERTIES OF CLAUSENA EXCAVATA AND MURRAYA KOENIGII**

WAN NOR IZZAH BT. WAN MOHAMAD ZAIN

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By

WAN NOR I'ZZAH BT. WAN MOHAMAD ZAIN

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
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Degree of Master of Science**

November 2005



As I was writing this thesis, I realized that without my parents I would not be here today. It is with this thought that I dedicate this thesis to my parents,

Hj. Wan Mohamad Zain bin Wan Yaakob

Hjh. Nik Khairiah binti Nik Abdul Rahman

in remembrance of their loves, guidance and sacrifices.

May Allah bless both of you.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in
fulfilment of the requirement for the degree of Master of Science

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Faculty : Medical and Health Sciences

The proximate composition of *Clausena excavata* and *Murraya koenigii* leaves, together with the vitamin and mineral contents were investigated. Studies on the antioxidant and antiproliferative properties of the plant extracts and essential oils were also carried out. The proximate analysis showed that *C. excavata* leaves contained higher moisture, ash and crude fibre contents compared to *M. koenigii*. The contents of vitamins A, C and E in *C. excavata* were found to be 47.78 mg/100 g, 586.30 mg/100 g and 267.67 mg/100 g, while in *M. koenigii* the results were 1406.32 mg/100 g, 374.38 mg/100 g and 18.52 mg/100 g respectively. It seems that *Murraya koenigii* contained higher zinc (0.09 mg/100 g sample), copper (0.1 mg/100 g sample), sodium (0.4 mg/100 g sample) and potassium (0.91 mg/100 g sample) compared to *Clausena excavata* that showed 0.01 mg zinc and copper per 100 g sample, sodium (0.37 mg/100 g sample) and potassium (0.73 mg/100 g sample). Iron (0.32 mg/100 g sample), magnesium (0.96 mg/100 g sample) and calcium (5.46 mg/100 g sample) were found to be higher in *C. excavata* than

M. koenigii that possessed 0.14 mg iron per 100 g sample, 0.76 mg magnesium per 100 g sample and 5.28 mg calcium per 100 g sample.

The essential oils were obtained by hydrodistillation using fresh leaves and analysed using GC-MS spectrometry. The leaf oil of *C. excavata* was mainly made up of safrole (89.85%) while the leaf oil of *M. koenigii* was mainly made up of β -farnesene (42.85%). Other components that were present in appreciable amounts in *M. koenigii* oil were naphthalene (12.17%), α -caryophyllene (8.09%), caryophyllene (5.47%) and eudesmol (4.34%).

The methanol and water crude extracts together with the essential oils of *C. excavata* and *M. koenigii* leaves were investigated for their antioxidant capacities in two different assays, namely the β -carotene bleaching method and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity. The methanol extract of *M. koenigii* showed the most potent antioxidant activity in β -carotene bleaching assay, giving a percentage of 86.13 %, while *C. excavata* showed 76.60 % in the assay. On the other hand, *M. koenigii* methanol extract showed weak effect in scavenging DPPH radical with an EC₅₀ value of 2.14 mg/ml, compared to the methanol extract of *C. excavata* which exhibited 0.89 mg/ml. The water extract of *C. excavata* showed higher antioxidant activity in both β -carotene bleaching method (76.02 %) and DPPH radical scavenging method (EC₅₀ value = 2.53 mg/ml) as compared to *M. koenigii* water extract which possessed 62.26 % antioxidant activity in β -

carotene bleaching method and 4.32 mg/ml EC₅₀ value in DPPH radical scavenging assay. Antioxidant activity of *M. koenigii* oil (91.01 %) was higher than *C. excavata* oil (66.3 %). Nevertheless, both of the essential oils did not present well in DPPH radical scavenging assay. The total phenolic content in the methanolic and water extracts of *C. excavata* and *M. koenigii* leaves, which were determined according to the Folin-Ciocalteu method, were expressed as gallic acid equivalents (GAEs); whereas the total phenolics in the methanolic extracts of both plants were higher than the water extracts. The methanolic extract of *C. excavata* had the highest phenolic content (103.33 mg of GAEs/g of sample extract) while *M. koenigii* methanol extract showed 63.92 mg of GAEs/g of sample extract. The total phenolic content of *M. koenigii* water extract possessed 53.62 mg of GAEs/g of sample extract while *C. excavata* exhibited 53.46 mg of GAEs/g of sample extract respectively.

HepG2 (hepatic cancer), MCF-7 (hormone-dependent breast cancer), MDA-MB-231 (non-hormone-dependent breast cancer), HeLa (cervical cancer) and CAOV3 (ovarian cancer) cell cultures were used to determine the antiproliferative activities of *C. excavata* and *M. koenigii*. The growth of viable cells was evaluated by using Microculture-tetrazolium (MTT) assay. Clausine-B, which was isolated from *C. excavata* was found to inhibit 50% of HeLa cancer cells' proliferation at 22.90 µg/ml, followed by *M. koenigii* methanol extract (25.00 µg/ml), *M. koenigii* essential oil (31.10 µg/ml) and *C. excavata* methanol extract (34.51 µg/ml). The *Clausena excavata* methanol

extract, water extract and essential oil were found to cause 50% cell death of MCF-7 cancer cell line at concentrations of 36.50, 95.00 and 59.00 $\mu\text{g/ml}$ respectively. Meanwhile, clausine-B and essential oil from *M. koenigii* were found to cause 50% cell death at 52.90 and 46.01 $\mu\text{g/ml}$ respectively. For HepG-2 liver cancer cell line, the highest mean total IC_{50} value could be seen in *M. koenigii* methanol extract which possessed 23.90 $\mu\text{g/ml}$. It was followed by clausine-B which was found to cause 50% cell death at a concentration of 28.94 $\mu\text{g/ml}$. The essential oil from *M. koenigii* and *C. excavata* methanol extract exhibited 48.00 and 53.03 $\mu\text{g/ml}$. Clausine-B and *M. koenigii* methanol extract were observed to inhibit the proliferation of MDA-MB-231 cell line at the concentrations of 21.50 and 37.98 $\mu\text{g/ml}$ respectively. Three samples were found to cause 50% cell death of CAOV3 which is the ovarian cancer cell line. The samples are clausine-B ($\text{IC}_{50} = 27.00$ $\mu\text{g/ml}$), *M. koenigii* methanol extract ($\text{IC}_{50} = 27.90$ $\mu\text{g/ml}$) and *C. excavata* methanol extract ($\text{IC}_{50} = 79.00$ $\mu\text{g/ml}$).

The findings of this study showed that the methanol extracts especially *M. koenigii* methanol extract have the great potential in antioxidant and antiproliferative activities. Clausine-B, was found to be active against all the cancer cell lines tested.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

**KOMPOSISI NUTRIEN, CIRI-CIRI ANTIOKSIDAN DAN
ANTIPROLIFERATIF *CLAUSENA ECAVATA* DAN *MURRAYA KOENIGII***

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November 2005

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Komposisi proksimat serta kandungan vitamin dan mineral bagi *Clausena excavata* dan *Murraya koenigii* dikaji. Kajian ke atas kandungan antioksidan dan antiproliferasi bagi ekstrak dan minyak pati bagi kedua-dua tumbuhan turut dilaksanakan. Analisis proksimat menunjukkan daun *C. excavata* mempunyai kandungan air, abu dan serat kasar yang lebih tinggi berbanding *M. koenigii*. Vitamin A, C dan E yang diperolehi bagi *C. excavata* adalah 47.78 mg/100 g, 586.30 mg/100 g dan 267.67 mg/100 g, manakala bagi *M. koenigii* keputusannya adalah 1406.32 mg/100 g, 374.38 mg/100 g dan 18.52 mg/100 g. Ia dapat dilihat bahawa *Murraya koenigii* mengandungi kandungan zink (0.09 mg/100 g sampel), kuprum (0.1 mg/100 g sampel), sodium (0.4 mg/100 g sampel) dan potasium (0.91 mg/100 g sampel) yang lebih tinggi berbanding dengan *Clausena excavata* yang menunjukkan 0.01 mg zink dan kuprum per 100 g sampel, sodium (0.37 mg/100 g sampel) dan potasium (0.73 mg/100 g sampel). Ferum (0.32 mg/100 g sampel), magnesium (0.96 mg/100 g sampel) dan kalsium (5.46 mg/100 g sampel)

didapati lebih tinggi di dalam *C. excavata* berbanding *M. koenigii* yang menunjukkan 0.14 mg ferum per 100 g sampel, 0.76 mg magnesium per 100 g sampel dan 5.28 mg kalsium per 100 g sampel.

Minyak pati diperolehi melalui teknik penyulingan hidro yang menggunakan daun segar dan dianalisis dengan menggunakan kaedah spektrometri GC-MS. Kandungan utama minyak pati daripada *C. excavata* adalah safrole (89.85%), manakala komponen utama bagi minyak pati daripada *M. koenigii* ialah β -farnesene (42.85%). Komponen-komponen lain yang diperolehi dalam amaun yang signifikan adalah naphthalene (12.17%), α -caryophyllene (8.09%), caryophyllene (5.47%) serta eudesmol (4.34%).

Kapasiti antioksidan bagi ekstrak kasar metanol dan air serta minyak pati daripada daun-daun *C. excavata* and *M. koenigii* dikaji melalui dua asai yang berbeza iaitu kaedah penurunan β -karotena dan aktiviti penghapusan radikal 1,1-difenil-2-pikrilhidrazil (DPPH). Ekstrak metanol *M. koenigii* menunjukkan aktiviti antioksidan yang paling berkesan di dalam asai penurunan β -karotena, dengan peratusan 86.13 %, manakala *C. excavata* menunjukkan 76.60 % di dalam asai tersebut. Namun begitu, ekstrak metanol *M. koenigii* adalah lemah di dalam penghapusan radikal DPPH dengan nilai $EC_{50} = 2.14$ mg/ml, berbanding ekstrak metanol *C. excavata* yang menunjukkan 0.89 mg/ml. Ekstrak air *C. excavata* mempamerkan aktiviti antioksidan yang tinggi di dalam kedua-dua kaedah penurunan β -

karotena (76.02 %) serta penghapusan radikal DPPH (nilai EC_{50} = 2.53 mg/ml) berbanding ekstrak air *M. koenigii*. Namun, aktiviti antioksidan minyak pati *M. koenigii* (91.01 %) adalah lebih tinggi jika dibandingkan dengan minyak pati *C. excavata* (66.3 %). Namun demikian, kedua-dua minyak pati terbabit tidak menunjukkan aktiviti yang berkesan di dalam asai penghapusan radikal DPPH. Jumlah kandungan fenolik bagi ekstrak metanol dan air daun *C. excavata* dan *M. koenigii* ditentukan melalui kaedah Folin-Ciocalteu, serta ditunjukkan dalam *gallic acid equivalent* (GAE); yang mana jumlah fenolik di dalam ekstrak metanol bagi kedua-dua tumbuhan adalah lebih tinggi berbanding ekstrak air. Ekstrak metanol *C. excavata* mengandungi jumlah fenolik yang paling tinggi (103.33 mg GAE/g ekstrak sampel) manakala ekstrak metanol menunjukkan 63.92 mg of GAE/g ekstrak sampel. Jumlah kandungan fenolik bagi ekstrak air *M. koenigii* memperlihatkan 53.62 mg GAE/g ekstrak sampel manakala *C. excavata* menunjukkan 53.46 mg GAE/g sampel ekstrak sampel.

Kultur sel HepG2 (kanser hepar), MCF-7 (kanser payudara bergantung hormon), MDA-MB-231 (kanser payudara tidak bergantung hormon), HeLa (kanser pangkal rahim) dan CAO3 (kanser ovari) telah digunakan untuk menentukan aktiviti antiproliferasi *C. excavata* dan *M. koenigii*. Pertumbuhan sel hidup ditentukan melalui asai Mikrokultur-tetrazolium (MTT). Clausine-B, yang diasingkan *C. excavata* telah didapati merencat 50% proliferasi sel kanser HeLa pada kepekatan 22.90 μ g/ml, diikuti dengan ekstrak metanol

M. koenigii (25.00 µg/ml), minyak pati *M. koenigii* (31.10 µg/ml) dan ekstrak metanol *C. excavata* (34.51 µg/ml). Ekstrak metanol, ekstrak air dan minyak pati *Clausena excavata*, telah dilihat menyebabkan kematian 50% sel bagi titisan sel kanser MCF-7 pada kepekatan-kepekatan berikut; 36.50, 95.00 dan 59.00 µg/ml. Manakala clausine-B dan minyak pati *M. koenigii* didapati menyebabkan kematian 50% sel pada kepekatan 52.90 dan 46.01 µg/ml. Bagi titisan sel kanser hepar HepG-2, jumlah purata nilai IC₅₀ dapat ditunjukkan di dalam ekstrak metanol *M. koenigii* yang mempamerkan 23.90 µg/ml. Ini diikuti dengan clausine-B yang didapati menyebabkan kematian 50% sel pada kepekatan 28.94 µg/ml. Minyak pati *M. koenigii* dan ekstrak metanol *C. excavata* memperlihatkan 48.00 dan 53.03 µg/ml. Clausine-B dan ekstrak metanol *M. koenigii* juga diperhatikan merencat proliferasi titisan sel kanser MDA-MB-231 pada kepekatan masing-masing 21.50 dan 37.98 µg/ml. Tiga sampel didapati menyebabkan kematian 50% sel CAO3 yang mana merupakan titisan sel kanser ovari. Sampel-sampel terbabit adalah clausine-B (IC₅₀ = 27.00 µg/ml), ekstrak metanol *M. koenigii* (IC₅₀ = 27.90 µg/ml) dan ekstrak metanol *C. excavata* (IC₅₀ = 79.00 µg/ml).

Hasil penemuan kajian ini mendapati ekstrak metanol terutamanya ekstrak metanol *M. koenigii* mempunyai potensi yang besar di dalam aktiviti antioksidan dan aktiviti antiproliferasi. Manakala clausine-B didapati aktif ke atas kesemua titisan sel kanser yang diuji.

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I certify that an Examination Committee has met on 8 November 2006 to conduct the final examination of Wan Nor I'zzah Bt. Wan Mohamad Zain on her Master of Science thesis entitled "Nutrient Composition, Antioxidant and Antiproliferative Properties of *Clausenia excavata* and *Murraya koenigi* " in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follows:

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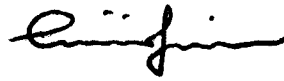
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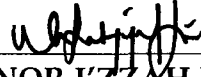


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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



WAN NOR IZZAH BT. WAN
MOHAMAD ZAIN

Date: **04 JAN 2006**

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LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectrophotometry
AOAC	Association of Official Analytical Chemists
ATCC	American Type of Culture Collection
BHT	butylated hydroxytoluene
CO ₂	carbon dioxide
DPPH	α, α -diphenyl- β -picrylhydrazyl
ECCC	European Collection of Cell Culture
EDX	Energy Dispersive X-ray
GAE	Gallic Acid Equivalent
g	gram
GC-MS	Gas Chromatography-Mass Spectroscopy
HCl	hydrochloric acid
HPLC	High Performance Liquid Chromatography
μ g	micro gram
μ l	micro litre
μ m	micro meter
l	litre
M	molar
ml	milli litre
mM	milli Molar
MeOH	methanol
min	minute
MTT	microculture tetrazolium
N	nitrogen
nm	nano meter
Na ₂ SO ₄	sodium sulfate
ppm	part per million
SEM	Scanning Electron Microscope
VPSEM	Variable Pressure Scanning Electron Microscope